General Rules for Designing PCR Primers, Xu lab

General Rules of Primer Design for **Restriction enzyme cloning**

A. Both forward primer and reverse primer should be in 5' to 3' direction;
B. 20~30 nucleotides in length, ideally;
C. 40~60% GC content, ideally
D. Melting temperature should be within 55^{o}C to 65 ^{o}C.
E. The 3' end (at least 16 bps) of both primer should be the direct repeat of your target gene, which we call them priming sequence;
F. The 5' end, upstream of the priming sequence, you should add the restriction enzyme sites (typically 6-letter nucleotides);
G. Upstream (5') of the restriction enzyme site, you should have 5 additional protection sequence (any sequence like aaggc), to increase the RE cutting efficiency
H. T_m's of forward and reverse primers must be similar (4^{o}C differences tolerated)
I. Primers cannot form secondary structure or stem-loops upon themselves
J. No primer-dimers greater than 6 bp between each primer and itself and also between the two primers.
K. The 3' end of the primer should be a G or a C.
L. Your primer will look like this
5'>5_bp_protection_sequence / 6bp RE site / your priming sequence>3'

General Rules of Primer Design for **Gibson Assembly**

A. Both forward primer and reverse primer should be in 5' to 3' direction;
B. 40~60 nucleotides in length, ideally;
C. 40~60% GC content, ideally;
D. Melting temperature should be within 50^{o}C to 70 ^{o}C;
E. The 3' end (at least 22 bps) of both primer should be the direct repeat of your target gene, which we call them priming sequence;
F. The 5' end, upstream of the priming sequence, you should add a homologous arm (HA) with length at least 28bp, up to 35bp; this HA **should be the direct repeat of your vector backbone**; forward primer design is easy; for reverse primer, make sure you know how to get the reverse complementary sequence from the DNA template.
G. T_m's of forward and reverse primers must be similar (4^{o}C differences tolerated)
H. Primers cannot form secondary structure or stem-loops upon themselves
I. No primer-dimers greater than 6 bp between each primer and itself and also between the two primers.
J. The 3' end of the primer should be a G or a C.
K. Your primer will look like this
5'>-28 to 35bp HA region / your priming sequence>3'

General Rules of Primer Design for **Mutagenic PCR**
Follow the protocol on the Primer X website http://www.bioinformatics.org/primerx/

Our IDT quotation number is 121178, send this Quote # to Victor when you place a primer order. This will give us half discount for all of the primers.